The Cytopathic Effect of Herpes Simplex Virus on HEp-2 Cells as shown by Scanning Electron Microscopy

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SUMMARY

The c.p.e. of herpes simplex virus was studied by scanning electron microscopy. The infected cells showed changes in size, shape, numbers of microvilli, numbers and integrity of intercellular bridges, and surface of the monolayer. When substantiated, these alterations may prove to represent the early phases of cellular reaction to virus invasion.

The HEp-2 cell is a human epithelial cell line derived from laryngeal squamous cell carcinoma, and can be infected readily by herpes simplex virus. This c.p.e. has been classically described (Gray, Tokumaru & Scott, 1958; Pereira, 1961; Horstmann & Hsiung, 1965). The c.p.e. is usually apparent 24 to 48 h after inoculation, and is currently a mainstay method of diagnostic virology. To observe the fine surface cellular changes that characterize the c.p.e., we have used the scanning electron microscope, inoculating and incubating the cell cultures by standard techniques (Lennette & Schmidt, 1969) to facilitate comparison between our findings and those by classic light microscopy. The surface changes noted may prove to represent the early phases of cellular reaction to virus invasion.

HEp-2 cells were seeded into sterile culture chambers, each containing a 12 mm round glass coverslip. These coverslips were used to culture the cells, and later as a substratum for insertion into the scanning electron microscope. Eagle's growth medium, in Earle's balanced salt solution (BSS), with 100 µg/ml penicillin and streptomycin, 0.25 µg/ml amphotericin B, and 10% foetal calf serum, was added to these cells, which were then incubated at 35 °C under 5% CO₂ atmosphere for 5 to 7 days until a confluent monolayer had formed on the coverslip. The preparations were then inoculated with 0.5 ml herpes simplex type 2 virus (HSV) in Hank's BSS at serial dilutions of 1.0 TCD₅₀ and 0.1 TCD₅₀. Replicate control preparations were inoculated with 0.5 ml sterile Hank's BSS in place of the virus. The adsorption of virus was continued for 1 h at room temperature; then 1.5 ml Eagle's growth medium was added. Incubation was continued thereafter for 24 h at 35 °C. At the end of the incubation period, the coverslips were washed in isotonic sodium cacodylate buffer (0.15 M) and fixed. After trying various fixation methods (2.5% glutaraldehyde in 0.1 M-sodium cacodylate followed by post-fixation in 1% OsO₄ in 0.15 M-sodium cacodylate), we settled on the following: 24 h fixation in 1% glutaraldehyde in 0.15 M-sodium cacodylate with added CaCl₂ and MgCl₂, followed by post-fixation in 1% OsO₄ in 0.15 M-sodium cacodylate (Boyde & Vesely, 1972; A. Boyde, personal communication). The fixing and washing procedures were completed in the culture chamber to minimize air drying in the transfer of the specimens.

After fixation, the cultures were washed in 0.15 M-sodium cacodylate buffer and dehydrated in ethyl alcohol and freon 113 in a sequence of incrementing dilution baths, each of approx. 10 min duration. The specimens were critical-point dried from freon 113 (Cohen, Marlow & Garner, 1968; Lewis & Nemanic, 1973), then coated with approx. 100 Å of...
The infected cells differed in several respects from uninfected cells (Fig. 1). They varied in shape and size, ranging from spherical cells less than 15 \( \mu \text{m} \) to flat squamous cells 50 to 60 \( \mu \text{m} \) across the longest axis (Fig. 2). The large forms contained more than one nuclear prominence, suggesting the coalescence of cells (Fig. 2). These enlarged cells were flat; whereas the small cells were round. The number of small round cells increased with the titre of the virus. The presence of these small and large cells disrupted the homogeneity of the infected monolayer. Such rounding-up of cells or parts of cells is the basis of the diagnosis of virus infection by light microscopy.

The uninfected cells were more uniform in size and measured approx. 15 to 25 \( \mu \text{m} \) across the longest axis. The entire surface of the cell was abundantly covered with microvilli. The microvilli in the uninfected cells were similar in size to those of the infected cells (0.07 to 0.1 \( \mu \text{m} \) diam.), and also similar to the microvilli observed in other cell lines in culture (Fisher & Cooper, 1967; Boyde, Weiss & Vesely, 1972). Their number appeared to diminish with the progressive increase in titre of the virus (8 microvilli per 100 sq. micron in the 1.0 TCD\(_{50}\) inoculation). The density of microvilli in the control non-infected culture was 250 to 300 microvilli per 100 sq. micron.

The bridging filaments were disrupted in the infected monolayer. This tendency to cleavage of the intercellular filaments increased with the titre of the virus. The uninfected cells had numerous fibrils or filaments (0.2 \( \mu \text{m} \) in diam.) joining adjacent cells (Fig. 1).

Cell monolayers were deliberately infected at low virus cell multiplicity to permit cell surface observation of the evolution of c.p.e. The marked changes seen in cell size and shape are readily discernible by scanning electron microscopy and correspond to the classic...
light microscopic description of c.p.e. (Pereira, 1961). The bridging intercellular filaments, approx. 0.2 μm in diam., are at the limiting resolution of the light microscope. The smaller diam. microvilli are beyond resolution of normal light microscopy. The changes we observed in the microvilli and intercellular bridges have not been previously described.

The large cells which apparently have more than one nuclear prominence (Fig. 2), correlate with the light microscope observations of Barski & Robineaux (1959) who described the formation of giant cells using phase contrast microcinematography. They considered that the adjacent cells coalesced to form the giant cells. The opposing cell membranes were broken and their cytoplasm merged. Our observation is that the cell membrane is altered, which may be preliminary to the coalescence. A contrary consideration is that the virus may prevent cells in mitosis from dividing, giving rise to multinucleated cells.

Cells have been synchronized and observed in the different phases of the cell cycle (Howard & Pelc, 1953; Porter, Prescott & Frye, 1973) have described, in a synchronized culture of Chinese hamster ovary cells (CHO), that cells during the synthesis of DNA in the S phase, flatten and lose their microvilli.

Cells round up during mitosis in synchronized cultures. This is the basis of the mitotic
selection of Tobey, Anderson & Petersen (1967), in which the round cells have reduced adhesion to the substratum. As the cells round up, one could expect intercellular connections between flat cells to cleave (Fig. 2).

We have previously seen increased numbers of pairs and quartets of cells whose relationships suggest that the cells have completed or are in the process of cytokinesis (Enlander et al. 1973). We believe that the loss of microvilli, cleavage of intercellular filaments and changes in cell shape seen in this study may reflect a maturation arrest in the infected cell prior to recovery or cell death.

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REFERENCES


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